

STIC-Biotech/ChemLib

53691

**From:** Ramirez, Delia  
**Sent:** Wednesday, October 24, 2001 4:26 PM  
**To:** STIC-Biotech/ChemLib  
**Subject:** search 09/488265

Hi,

I would like to request the following searches (appl. 09/488265, Lehman et al.)

1. a standard search of seq id 25, 28, 30 in the nucleic acid databases (interference)
2. a standard search of seq id 26, 27, 29, 31 in the protein databases (interference)

Thanks,

Delia M. Ramirez, Ph.D.  
Patent Examiner - Art Unit 1652 -Mail room 10C01  
USPTO  
1911 S. Clark Street, Crystal Mall 1, 4E18  
Arlington, VA 22202  
(703) 306-0288  
delia.ramirez@uspto.gov

*Edward S. Hart*  
*Patent Examiner - Art Unit 1652*  
*USPTO*  
*1911 S. Clark St. Arlington, VA 22202*  
*Phone: 306-9203*

P

# SEARCH REQUEST FORM

Scientific and Technical Information Center

Access DB#

53691

Requester's Full Name: \_\_\_\_\_ Examiner #: \_\_\_\_\_ Date: \_\_\_\_\_  
Art Unit: \_\_\_\_\_ Phone Number 30 \_\_\_\_\_ Serial Number: \_\_\_\_\_  
Mail Box and Bldg/Room Location: \_\_\_\_\_ Results Format Preferred (circle): PAPER DISK E-MAIL

If more than one search is submitted, please prioritize searches in order of need.

\*\*\*\*\*  
Please provide a detailed statement of the search topic, and describe as specifically as possible the subject matter to be searched. Include the elected species or structures, keywords, synonyms, acronyms, and registry numbers, and combine with the concept or utility of the invention. Define any terms that may have a special meaning. Give examples or relevant citations, authors, etc., if known. Please attach a copy of the cover sheet, pertinent claims, and abstract.

Title of Invention: \_\_\_\_\_

Inventors (please provide full names): \_\_\_\_\_

Earliest Priority Filing Date: \_\_\_\_\_

*\*For Sequence Searches Only\* Please include all pertinent information (parent, child, divisional, or issued patent numbers) along with the appropriate serial number.*

## STAFF USE ONLY

	Type of Search	Vendors and cost where applicable
Searcher _____	NA Sequence (#) <u>3</u>	STN _____
Searcher Phone # _____	AA Sequence (#) <u>4</u>	Dialog _____
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PTC Form 100-1000

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**Search Results - Record(s) 1 through 3 of 3 returned.**☐ 1. Document ID: US 6153418 A

L3: Entry 1 of 3

File: USPT

Nov 28, 2000

US-PAT-NO: 6153418

DOCUMENT-IDENTIFIER: US 6153418 A

TITLE: Consensus phytases

DATE-ISSUED: November 28, 2000

## INVENTOR-INFORMATION:

NAME

CITY

STATE

ZIP CODE

COUNTRY

Lehmann; Martin

Inzlingen

DEX

US-CL-CURRENT: 435/195; 424/94.1, 424/94.6, 435/196

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw. Desc	Image
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☐ 2. Document ID: EP 897985 A2

L3: Entry 2 of 3

File: EPAB

Feb 24, 1999

PUB-NO: EP000897985A2

DOCUMENT-IDENTIFIER: EP 897985 A2

TITLE: Consensus phytases

PUBN-DATE: February 24, 1999

## INVENTOR-INFORMATION:

NAME

COUNTRY

LEHMANN, MARTIN

DE

INT-CL (IPC): C12N 15/55; C12N 9/16; C12N 9/00; A23L 1/03; A23K 1/165; A61K 38/46  
EUR-CL (EPC): C12N009/16

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw. Desc	Image
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☐ 3. Document ID: AU 200020928 A, WO 200043503 A1

L3: Entry 3 of 3

File: DWPI

Aug 7, 2000

DERWENT-ACC-NO: 2000-491161  
DERWENT-WEEK: 200055  
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TITLE: Novel phytases with improved properties such as temperature stability, pH stability and substrate specificity, for use in pharmaceuticals and compound foods and feeds

INVENTOR: LEHMANN, M

PRIORITY-DATA: 1999DK-0001340 (September 21, 1999), 1999DK-0000092 (January 22, 1999)

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200020928 A	August 7, 2000		000	C12N009/16
WO 200043503 A1	July 27, 2000	E	238	C12N009/16

INT-CL (IPC): C12N 9/16

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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Terms

Documents

phytase? and lehman?

3

Display

10

Documents, starting with Document:

3

Display Format:

CIT

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=> d his

(FILE 'HOME' ENTERED AT 16:30:51 ON 24 OCT 2001)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR,  
AQUASCI,

BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO,  
CABA,

CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,  
DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 16:31:02  
ON

24 OCT 2001

SEA PHYTAS?

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486 FILE AGRICOLA  
12 FILE ANABSTR  
33 FILE AQUASCI  
346 FILE BIOBUSINESS  
32 FILE BIOCOMMERCE  
1149 FILE BIOSIS  
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170 FILE BIOTECHNO  
916 FILE CABA  
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2 FILE HEALSAFE  
 99 FILE IFIPAT  
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 160 FILE LIFESCI  
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 380 FILE PASCAL  
 66 FILE PHIN  
 127 FILE PROMT  
 844 FILE SCISEARCH  
 129 FILE TOXLIT  
 435 FILE USPATFULL  
 190 FILE WPIDS  
 190 FILE WPINDEX  
 3 FILE NAPRALERT  
 44 FILE NLDB

L1 QUE PHYTAS?

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FILE 'CAPLUS, DGENE, BIOSIS, CABA, SCISEARCH, AGRICOLA, USPATFULL,  
 MEDLINE, PASCAL, BIOBUSINESS, GENBANK, FSTA, FROSTI, EMBASE,  
 BIOTECHDS,  
 ESBIODBASE, WPIDS, BIOTECHNO, LIFESCI, TOXLIT, PROMT, JICST-EPLUS, IFIPAT,  
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L2 10623 S PHYTAS?  
 L3 5507 DUP REM L2 (5116 DUPLICATES REMOVED)  
 L4 5507 S L3 AND PHYTAS?  
 L5 0 S L4 AND LEHMA?  
 L6 0 S L4 AND LEHMAN?  
 L7 178 S L4 AND MARTIN?

=> s l4 and lassen?

L8 1 L4 AND LASSEN?

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NEWS 5 Apr 23 Search Derwent WPINDEX by chemical structure  
NEWS 6 Apr 23 PRE-1967 REFERENCES NOW SEARCHABLE IN CAPLUS AND CA  
NEWS 7 May 07 DGENE Reload  
NEWS 8 Jun 20 Published patent applications (A1) are now in USPATFULL  
NEWS 9 JUL 13 New SDI alert frequency now available in Derwent's  
DWPI and DPCI  
NEWS 10 Aug 23 In-process records and more frequent updates now in  
MEDLINE  
NEWS 11 Aug 23 PAGE IMAGES FOR 1947-1966 RECORDS IN CAPLUS AND CA  
NEWS 12 Aug 23 Adis Newsletters (ADISNEWS) now available on STN  
NEWS 13 Sep 17 IMSworld Pharmaceutical Company Directory name change  
to PHARMASEARCH  
NEWS 14 Oct 09 Korean abstracts now included in Derwent World Patents  
Index  
NEWS 15 Oct 09 Number of Derwent World Patents Index updates increased  
NEWS 16 Oct 15 Calculated properties now in the REGISTRY/ZREGISTRY File  
NEWS 17 Oct 22 Over 1 million reactions added to CASREACT  
NEWS 18 Oct 22 DGENE GETSIM has been improved  
  
NEWS EXPRESS August 15 CURRENT WINDOWS VERSION IS V6.0c,  
CURRENT MACINTOSH VERSION IS V6.0 (ENG) AND V6.0J (JP),  
AND CURRENT DISCOVER FILE IS DATED 07 AUGUST 2001  
  
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ENTRY	SESSION
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FULL ESTIMATED COST

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI,  
BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA,  
CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB,  
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62 FILES IN THE FILE LIST IN STNINDEX

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=> s phytas?

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49 FILES HAVE ONE OR MORE ANSWERS, 62 FILES SEARCHED IN STNINDEX

L1 QUE PHYTAS?

=> d rank

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=> s phytas?  
L2 10623 PHYTAS?

=> dup rem l2  
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ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE  
PROCESSING IS APPROXIMATELY 9% COMPLETE FOR L2  
PROCESSING IS APPROXIMATELY 37% COMPLETE FOR L2  
PROCESSING IS APPROXIMATELY 53% COMPLETE FOR L2  
PROCESSING IS APPROXIMATELY 66% COMPLETE FOR L2  
PROCESSING IS APPROXIMATELY 78% COMPLETE FOR L2  
PROCESSING IS APPROXIMATELY 87% COMPLETE FOR L2  
PROCESSING IS APPROXIMATELY 95% COMPLETE FOR L2  
PROCESSING COMPLETED FOR L2  
L3 5507 DUP REM L2 (5116 DUPLICATES REMOVED)

=> s l3 and phytas?  
4 FILES SEARCHED...  
14 FILES SEARCHED...  
24 FILES SEARCHED...  
L4 5507 L3 AND PHYTAS?

=> s l4 and lehma?  
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L6 0 L4 AND LEHMAN?

=> s l4 and martin?  
L7 178 L4 AND MARTIN?

=> d ti 1-178

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SEA PHYTAS?

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486 FILE AGRICOLA  
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L1 QUE PHYTAS?  
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FILE 'CAPLUS, DGENE, BIOSIS, CABA, SCISEARCH, AGRICOLA, USPATFULL,  
 MEDLINE, PASCAL, BIOBUSINESS, GENBANK, FSTA, FROSTI, EMBASE, BIOTECHDS,  
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L2 10623 S PHYTAS?  
 L3 5507 DUP REM L2 (5116 DUPLICATES REMOVED)  
 L4 5507 S L3 AND PHYTAS?  
 L5 0 S L4 AND LEHMA?  
 L6 0 S L4 AND LEHMAN?  
 L7 178 S L4 AND MARTIN?

=> d 17 1-178 ti

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=> d 1-10

L7 ANSWER 1 OF 178 USPATFULL  
 AN 2001:185559 USPATFULL  
 TI Hybrid maize plant & seed 34T38  
 IN Whitaker, David Walter, Narvon, PA, United States  
 PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.  
 corporation)  
 PI US 6307132 B1 20011023  
 AI US 1999-376036 19990817 (9)  
 DT Utility  
 FS GRANTED  
 LN.CNT 1664  
 INCL INCLM: 800/320.100  
 INCLS: 800/298.000; 800/275.000; 800/271.000; 800/268.000; 800/266.000;  
 800/301.000; 800/302.000; 800/303.000; 435/412.000; 435/424.000;  
 435/430.000; 435/430.100  
 NCL NCLM: 800/320.100  
 NCLS: 800/298.000; 800/275.000; 800/271.000; 800/268.000; 800/266.000;

800/301.000; 800/302.000; 800/303.000; 435/412.000; 435/424.000;  
435/430.000; 435/430.100

IC [7]

ICM: A01H005-00

ICS: A01H004-00; A01H001-00; C12N005-04

EXF 800/320.1; 800/298; 800/275; 800/271; 800/268; 800/266; 435/412;  
435/422; 435/430; 435/430.1

L7 ANSWER 2 OF 178 USPATFULL

AN 2001:185558 USPATFULL

TI Soybean variety 93B66

IN Corbin, Thomas C., Monticello, IL, United States

Freestone, Robert E., Cedar Falls, IA, United States

PA Pioneer Hi-Bred International, Inc., United States (U.S. corporation)

PI US 6307131 B1 20011023

AI US 2000-500903 20000209 (9)

DT Utility

FS GRANTED

LN.CNT 1456

INCL INCLM: 800/312.000

INCLS: 800/260.000; 800/265.000; 800/266.000; 800/267.000; 800/268.000;  
800/278.000; 800/300.000; 435/415.000; 435/419.000; 435/421.000;  
435/426.000; 435/430.000; 435/430.100; 435/468.000

NCL NCLM: 800/312.000

NCLS: 800/260.000; 800/265.000; 800/266.000; 800/267.000; 800/268.000;  
800/278.000; 800/300.000; 435/415.000; 435/419.000; 435/421.000;  
435/426.000; 435/430.000; 435/430.100; 435/468.000

IC [7]

ICM: A01H001-04

ICS: A01H005-00; A01H005-10; A01H004-00; C12N005-04

EXF 800/260; 800/265; 800/266; 800/267; 800/268; 800/278; 800/300; 800/312;  
435/415; 435/419; 435/421; 435/426; 435/430; 435/430.1; 435/468

L7 ANSWER 3 OF 178 USPATFULL

AN 2001:185550 USPATFULL

TI Methods and compositions for transgene identification

IN Kriz, Alan L., Gales Ferry, CT, United States

Spencer, T. Michael, Mystic, CT, United States

PA Dekalb Genetics Corporation, Dekalb, IL, United States (U.S.  
corporation)

PI US 6307123 B1 20011023

AI US 1998-80625 19980518 (9)

DT Utility

FS GRANTED

LN.CNT 5135

INCL INCLM: 800/282.000

INCLS: 800/266.000; 800/288.000; 800/300.000; 800/301.000; 536/023.400;  
536/024.100

NCL NCLM: 800/282.000

NCLS: 800/266.000; 800/288.000; 800/300.000; 800/301.000; 536/023.400;  
536/024.100

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ICM: C12N015-00

ICS: C12M015-09; A01H005-00; A01H001-02

EXF 536/24.1; 536/23.4; 435/69.1; 435/172.3; 435/410; 435/411; 435/419;  
435/430.1; 800/278; 800/266; 800/282; 800/288; 800/200; 800/301

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 4 OF 178 USPATFULL

AN 2001:179335 USPATFULL

TI Hybrid maize plant & seed 39n03

IN Kramer, Joachim Ernst, Neusiedl, Austria

PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.

corporation)  
PI US 6303851 B1 20011016  
AI US 2000-519974 20000307 (9)  
RLI Continuation of Ser. No. US 1999-258554, filed on 26 Feb 1999, now  
abandoned  
DT Utility  
FS GRANTED  
LN.CNT 1603  
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ICM: A01H005-00  
ICS: A01H004-00; A01H001-00; C12N005-04  
EXF 800/320.1; 800/298; 800/275; 800/271; 800/268; 800/266; 800/300-303;  
435/412; 435/424; 435/430; 435/430.1

L7 ANSWER 5 OF 178 USPATFULL  
AN 2001:170893 USPATFULL  
TI Methods and compositions for the introduction of molecules into cells  
IN Held, Bruce Marvin, Ames, IA, United States  
Wilson, Herbert Martin, Ames, IA, United States  
Hou, Liming, St. Louis, MO, United States  
Lewnau, Carol Jean, Ames, IA, United States  
Eby, Janelle Christine, Ames, IA, United States  
PI US 2001026941 A1 20011004  
AI US 2001-850476 A1 20010507 (9)  
RLI Continuation of Ser. No. US 1999-450226, filed on 29 Nov 1999, PENDING  
DT Utility  
FS APPLICATION  
LN.CNT 1907  
INCL INCLM: 435/468.000  
NCL NCLM: 435/468.000  
IC [7]  
ICM: C12N015-82

L7 ANSWER 6 OF 178 USPATFULL  
AN 2001:168312 USPATFULL  
TI Hybrid maize plant and seed 35H53  
IN Chapman, Michael A., Madison Lake, MN, United States  
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.  
corporation)  
PI US 6297433 B1 20011002  
AI US 2000-489136 20000121 (9)  
DT Utility  
FS GRANTED  
LN.CNT 2093  
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INCLS: 800/298.000; 800/275.000; 800/271.000; 800/268.000; 800/301.000;  
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ICM: A01H005-00  
ICS: A01H004-00; A01H001-00; C12X005-04

EXF 800/320.1; 800/298; 800/275; 800/271; 800/268; 800/301; 800/302;  
800/303; 435/412; 435/424; 435/430; 435/430.1

L7 ANSWER 7 OF 178 USPATFULL  
AN 2001:168311 USPATFULL  
TI Hybrid maize plant and seed 39A26  
IN Puskaric, Vladimir, Woodstock, Canada  
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S. corporation)  
PI US 6297432 B1 20011002  
AI US 1999-259868 19990301 (9)  
DT Utility  
FS GRANTED  
LN.CNT 1989  
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ICM: A01H005-00  
ICS: A01H004-00; A01H001-00; C12N005-04  
EXF 800/320.1; 800/298; 800/278; 800/271; 800/268; 800/266; 435/412;  
435/424; 435/430; 435/430.1

L7 ANSWER 8 OF 178 USPATFULL  
AN 2001:158041 USPATFULL  
TI Alkaline protease deficient filamentous fungi  
IN Lehmbeck, Jan, Veks.o slashed., Denmark  
PA Novo Nordisk A/S Novo alle, Bagsvaerd, Denmark (non-U.S. corporation)  
PI US 6291209 B1 20010918  
AI US 1998-148751 19980904 (9)  
RLI Continuation of Ser. No. WO 1997-DK135, filed on 26 Mar 1997  
PRAI DK 1996-354 19960327  
DK 1996-555 19960509  
DT Utility  
FS GRANTED  
LN.CNT 644  
INCL INCLM: 435/069.100  
INCLS: 435/484.000; 435/254.300; 435/320.100  
NCL NCLM: 435/069.100  
NCLS: 435/484.000; 435/254.300; 435/320.100  
IC [7]  
ICM: C12N015-64  
EXF 435/6; 435/69.1; 435/183; 435/194; 435/320.1; 435/254.21; 435/254.3;  
435/256.1; 435/477; 435/484  
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L7 ANSWER 9 OF 178 USPATFULL  
AN 2001:153197 USPATFULL  
TI Hybrid maize plant and seed 36D14  
IN Gogerty, Joseph Kevin, Algona, IA, United States  
PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S. corporation)  
PI US 6288310 B1 20010911  
AI US 1999-259989 19990301 (9)  
DT Utility  
FS GRANTED  
LN.CNT 1800  
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NCL NCLM: 800/320.100  
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 ICM: A01H005-00  
 ICS: A01H004-00; A01H001-00; C12H005-04  
 EXF 800/320.1; 800/298; 800/275; 800/271; 800/301-303; 435/412; 435/424;  
 435/430; 435/430.1  
 L7 ANSWER 10 OF 178 USPATFULL  
 AN 2001:148156 USPATFULL  
 TI Hybrid maize plant and seed 32Y52  
 IN Colbert, Terry R., Fort Branch, IN, United States  
 PA Pioneer Hi-Bred International, Inc., Des Moines, IA, United States (U.S.  
 corporation)  
 PI US 6284953 B1 20010904  
 AI US 1999-237518 19990126 (9)  
 DT Utility  
 FS GRANTED  
 LN.CNT 1871  
 INCL INCLM: 800/320.100  
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 ICM: A01H004-00  
 ICS: A01H005-00; A01H003-22; C12N015-29  
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SEA PHYTAS?

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L8 ANSWER 1 OF 1 PROMT COPYRIGHT 2001 Gale Group

ACCESSION NUMBER: 2001:356673 PROMT  
 TITLE: New strategies in supplementation of phosphorus discussed.  
 AUTHOR(S): BRUYNE, KARL DE  
 SOURCE: Feedstuffs, (6 Mar 2000) Vol. 72, No. 10, pp. 11.  
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L8 ANSWER 1 OF 1 PROMT COPYRIGHT 2001 Gale Group  
AN 2001:356673 PROMT  
TI New strategies in supplementation of phosphorus discussed.  
AU BRUYNE, KARL DE  
SO Feedstuffs, (6 Mar 2000) Vol. 72, No. 10, pp. 11.  
ISSN: 0014-9624.  
PB Miller Publishing Company, Inc.  
DT Newsletter  
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WC 3938  
TX ABSTRACT

This paper presents several aspects of the use of **phytase** in swine and poultry diets, including the effect of **phytase** on other minerals, amino acids, water consumption and stability. Considerations for the inclusion of modern feed phosphates are also discussed.

**Phytase** increases digestibility of vegetal phosphorus and calcium. It is well documented that **phytase** increases digestibility of both phosphorus and calcium. Dutch research established in the beginning of the 1990s (Jongbloed, 1993) a **phytase** activity equivalence to feed phosphates of 500 IU **phytase** = 1 g phosphorus from monocalcium phosphate or 1.1 g phosphorus from dicalcium phosphate dihydrate for pigs. At higher doses, efficacy decreases. This equivalency has been taken over by most **phytase** producers in their recommendations. It is based on a digestibility coefficient for monocalcium phosphate of 80%.

Eeckhout (1997), however, found a digestibility for monocalcium phosphate of 93%. It is also assumed that this equivalency is valid independent of the raw material used. However, literature indicates clearly that the improvement of phosphorus digestibility by **phytase** varies clearly in function of the raw material.

It is most probable that the amount and type of phytic phosphorus and the presence of vegetal **phytase** influences the performance of the microbial **phytase**; it is best when a lot of phytic phosphorus is present (Rodehutsord and Dungelhoeft, 1994). Using microbial **phytase**, they improved phosphorus digestibility of corn from 18 to 56%. The phosphorus digestibility of wheat improved from 62 to 74%, while improvement in triticale was from 52 to 67%. The improvement in digestibility is much higher in corn. This indicates that the presence of vegetal **phytase** hydrolyses phytic phosphorus and eliminates substrate for the microbial **phytase**, thus reducing its efficacy.

Another explanation may be the solubility of corn phytate is higher than the solubility of wheat phytate at pH 4-7 (Scheuermann, 1988). Therefore, it is very improbable that the Dutch value, obtained mainly on **phytase**-poor raw materials, is applicable everywhere. It is clear that in countries where a lot of cereals (which contain endogenous **phytase**) are used, the above mentioned equivalency is not valid but should be 700 IU **phytase** = 1 g phosphorus from monocalcium phosphate. In non-pelleted, cereal-rich compound feeds, it is not economical to use microbial **phytase**.

Kemme (1997) came to the conclusion that this equivalency was not valid for every kind of pig and also varied with the age of the pig. Indeed, for

piglets, 500 IU of **phytase** liberate only 0.66 g of digestible phosphorus. He even measured differences in sows at the beginning or end of pregnancy, finding 0.32 and 0.74 g of digestible phosphorus for 500 IU of **phytase**, respectively. This is another factor of variability which complicates formulation with **phytase**.

**Phytase** increases digestibility of some amino acids. Several researchers have reported that **phytase** improves ileal digestibility of amino acids and nitrogen both in pigs and poultry (Officer and Batterham, 1992; Mroz et al., 1994; Yi et al., 1994; Farrell et al., 1993; Van de Klis and Versteegh, 1991). In turkeys, Yi et al. (1996) found an improved ileal digestibility for some amino acids, but not for cystine and methionine. Sebastian (1997) also found only a partial effect on amino acid digestibility in male and female broilers. Pallauf (1994) and Newkirk and Classen (1995) found no significant effect on crude protein digestibility. In spite of this possible increased amino acid digestibility, a significant positive effect on feed conversion ratio is seldom observed (Simons et al., 1990; Broz et al., 1994; Denbow et al., 1995; Sebastian, 1996). The possible effect of **phytase** on growth parameters needs to be confirmed. Application at this stage of this "protein effect" could lead to adverse effects. It would be useful to examine the factors causing the variability of the findings.

**Phytase** activity depends on calcium:phosphorus ratio. Excess dietary calcium has a negative effect on the utilization of phosphorus because of formation of insoluble calcium-phytate complexes (Nelson and Kirby, 1987). Since **phytase** liberates calcium, and thus increases the total amount of available calcium in the feed, it is clear that possible interactions between **phytase** and calcium content have to be monitored. It is also possible that high calcium levels increase pH to levels that are not optimal for **phytase** activity. Liu et al. (1997) found lowering the calcium:phosphorus ratio from 1.5:1.0 to 1.0:1.0 in pigs increased most investigated parameters, except bone breaking strength. Lantzsich et al. (1995) found maximum phosphorus retention at a calcium:phosphorus ratio of 1.5:1.0. Quian et al. (1997) found 1.25:1.0 as optimal ratio. In poultry, some authors (Sebastian, 1996; Schoner, 1993) found improved growth and mineral utilization at lower calcium levels. Huyghebaert (1996) found a higher incidence of tibial dyschondroplasia (TD) at narrow calcium:phosphorus ratios. It seems that the existing calcium recommendations have to be evaluated in view of **phytase** utilization.

**Phytase** increases water consumption and causes wet manure. For broilers, it has been proven (Schutte, 1993) that the replacement of 0.5% monocalcium phosphate by 500 IU of microbial **phytase** results in significant increase of water intake and the water:feed ratio 4-5%. There are indications that this is caused by an increase of magnesium and calcium availability, and perhaps also of sodium and potassium. These are all minerals that highly increase the water metabolism and the degree of moisture in excreta. This indirect negative aspect of the application of microbial **phytase** needs to be taken into consideration if one wants to prevent problems with wet droppings and litter.

**Phytase** gives poorer bone mineralization. As to bone mineralization in poultry, it seems difficult to obtain maximum bone strength or ash content by supplementation of microbial **phytase**, even in doses of 1,000 IU/kg (Sauveur, 1993; Simons, 1993; Cromwell, 1995; Cantor, 1996; Liu et al., 1997) in comparison with the control diet (0.73% phosphorus, 0.45% available phosphorus; Huyghebaert, 1992). This is in spite of the fact that the phosphorus liberated by **phytase** seems to be utilized as efficiently for skeleton mineralization as the added phosphorus from dicalcium phosphate dihydrate. This is because the phosphorus available per animal does not always reach that of the control

diet with exclusively mineral phosphorus supplementation (Simons, 1993; Huyghebaert, 1992).

**Phytase** activity is influenced by premix ingredients. Practical experience in Holland showed interference between some premix ingredients and microbial **phytase**. Notably, liquid choline and iron sulfate heptahydrate caused important loss of activity after storage. It is mainly the presence of moisture that causes the interaction with the enzyme. It is very probable that minerals with an important content of molasses will also interact with the microbial **phytase**, which will lead to activity loss. This seems to make the use of **phytase** in mineral feed impossible.

**Phytase** is not thermo-stable. Eeckhout (1998) performed an extensive trial on the influence of steam pelleting and storage temperature on the recovery of **phytase**.

In a first experiment, the influence of hole diameter, channel length and feed composition on the **phytase** activity was measured. It is clearly indicated that all friction-enhancing factors have a negative influence on **phytase** stability (Figures 1,2 and 3). It appears that a die hole of 2 mm causes much more **phytase** activity loss than a die of 4mm. A longer channel length of 40 mm seems also to lower the activity of the **phytase**, mainly at lower conditioning temperatures. In this way, the channel length supplies, via friction, the additional temperature necessary to destroy the **phytase** activity. Feed composition also plays an important role on **phytase** stability. High-fat feeds, like broiler feed, go through the die with less friction and less temperature increase, and hence the recovery of activity is much higher than in the fiber-rich pig feeds.

In a second experiment, the influence of storage temperature on **phytase** activity has been measured. Figures 4 and 5 demonstrate that an additional loss of 20-40% **phytase** activity can be suffered after only two days of storage at 40 and 50[degrees]C, respectively.

In a third experiment, liquid **phytase** was used. Application of liquid **phytase** is difficult and requires an important investment. It sometimes can lead to heterogeneous dispersion of the **phytase** over the pellets. Another important factor is loss of activity due to pneumatic manipulations. Indeed, an important fraction of the **phytase** is applied on the tops of the pellets. As a result, a lot of **phytase** ends up in the meal fraction of the feed. This is often recycled and pelleted again. In this way, the sprayed **phytase** is destroyed. Creation of a meal fraction also happens outside the feed mill on the farm. During storage, the loss of activity of the liquid **phytase** was even greater than for the solid **phytase**. This is probably because the sprayed **phytase** is totally on the outside of the pellet. This experiment indicates that extreme attention has to be paid to the loss of **phytase** activity occurring during production and storage. At very high pelleting and storage temperatures, total destruction of **phytase** is not improbable (Figure 6). It is also shown that a simple rule of thumb accounting for 30% activity loss is not adequate since the major influences are raw materials, conditioning temperature and even die diameter and channel length.

In a fourth experiment, a "protected" **phytase** was tested. This type of **phytase** came on the market a few years after the original **phytase** and after the liquid **phytase**. The argument of the producer for this innovation was the inability of the original **phytase** to survive pelleting. In this way, our earlier

results were indirectly confirmed. In this experiment, we did the now common pelleting test with a 2 x 40 mm die (broiler feed) and a 4 x 50 mm die (piglet feed). Later on, these pellets were stored for two weeks at different temperatures.

The **phytase** recovery in the broiler feed shows an important reduction at 71[degrees]C conditioning temperature. At 82[degrees]C the recovery was 40%. For the piglet feed, comparable results were found; the recovery at high conditioning temperature (82[degrees]C) was 60%. The larger size of this pellet may explain a higher recovery (Figures 7 and 8).

Figures 9 and 10 show the results of the storage experiment. At 30[degrees]C the recovery was reduced to 85-80%. At higher storage temperatures, the loss increased up to 30 (at 40[degrees]C), and even to 60% (at 50[degrees]C). When, due to extreme storage conditions, the temperature in the outer parts of a bin increases, the activity loss may be very important.

The overall recovery of the granulated form shows a higher stability (higher recovery) than the classical **phytase**, which was almost totally lost. However, at 80[degrees]C the activity loss still is very important.

#### Feed phosphates

Environmental pressure and progress in livestock production resulted in the development of a more precise evaluation system. Started in the 1980s, a method was developed in the Netherlands to express the biological value of phosphorus in the feed as apparent digestible phosphorus by measuring the difference in ingested and excreted phosphorus.

In most countries, feed compounders are still expressing the biological value of raw materials as available phosphorus, in which only little differentiation is made. Therefore, it would be worthwhile to translate the outcome of the digestibility trials into these systems, enabling feed compounders to optimize the diets more closely to the needs of the animal. This would result in a reduction in the excretion of phosphorus into the environment without endangering the animal welfare or economic results.

Digestibility trials in pigs. Although the absolute digestibility levels differ between trials, the ranking between the different types of phosphorus is the same (Table 1). Most probably this is caused by differences in protocol and the statistical analysis applied. It is very important that, when carrying out a digestibility trial, the level of vegetal phosphorus in the basal feed is as low as possible. Otherwise, this phosphorus has a negative interaction with the feed phosphates tested, resulting in an underestimation.

For those reasons, Eeckhout and De Paepe (1997) slightly adapted the digestibility procedure. They used a semi-synthetic diet virtually free of phosphorus, 0.037%. There were two treatments per phosphate: 1 or 2 g added phosphorus/kg. Calcium was added at 5 g/kg. Liveweight of the animals was between 35 and 40 kg.

To counteract marker problems and the high variability of phosphorus digestibility, a total collection of feces in metabolic cages was applied over a 10-day period. In order to guarantee the accurate intake of the calculated amounts of phosphorus and calcium, the different feed phosphates and calcium carbonate were carefully weighed, per feeding and per pig, and hand mixed in the individual feeding troughs.

Results indicated the feed phosphates with the highest digestibility

coefficients have been underestimated in earlier trials. It is therefore suggested to use to the Eeckhout procedure (1997) for future digestibility trials.

Digestibility trials in poultry. Poultry and pigs are quite different with respect to the anatomy of their digestive system. Therefore, poultry and pigs digest feed phosphates quite differently. The trials mentioned in Table 2 were also done with synthetic vegetal phosphorus-free basal diets.

Between the two trials, the results are quite comparable. The ranking between the different types is more or less the same as found in the trials with pigs, although there are some differences in the absolute digestibility values. In particular, the value for dihydrated dicalcium phosphate is much higher with poultry than with pigs. Poultry seem to digest dihydrated dicalcium phosphate much better. Defluorinated phosphate and anhydrous dicalcium phosphate seem not to be suited for poultry.

Based on the above mentioned research data, we propose in Table 3 a digestibility coefficient for most commercial feed phosphates. These values can be used for evaluating feed phosphates or even for formulating feeds with digestible phosphorus constraints.

As a conclusion, it seems that dihydrated dicalcium phosphate and monocalcium phosphate are particularly suitable for poultry, whereas monocalcium and monodicalcium phosphate are preferred for pigs.

#### Conclusions

In spite of the fact that European livestock, although rather concentrated in some areas, is not confronted with the same environmental problems in every region, it seems worthwhile to pay some attention to phosphorus supply of the animals. As indicated, two main strategies exist. The first one is using **phytase**. This enzyme offers certainly new perspectives in reducing phosphorus excretion. The main disadvantage of this product is its variability. Its efficacy depends on the raw materials used in the feeds and is affected by production parameters such as premix ingredients, conditioning temperatures, die diameter, channel length, fiber content of the feed and storage temperatures. This makes it impossible for the feed compounder to predict its value and hence to work with **phytase** without using safety margins. One of the most important goals of **phytase**, avoiding excessive excretion of phosphorus in the manure, is in this way difficult to achieve. Since there is no danger for phosphorus saturation of most European soils, it is not useful to take unnecessary risks. It is certainly not necessary to take these risks since modern high-quality feed phosphates are sources of highly digestible phosphorus. In this way, they offer a stable, predictable supply of digestible phosphorus that is not affected by other raw materials, production parameters or storage.

Karl De Bruyne is area manager for Tessenderlo Chemie, Brussels, Belgium. This paper was submitted through EMFEMA, the European federation of producers of minerals for animal nutrition.

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#### Summary of apparent digestibility trials in pigs

	Holland	Denmark
Dicalcium phosphate	65	52
Dicalcium phosphate dihydrate, Aliphos	69	59
Dicalcium phosphate dihydrate, ex gelatine	87	--
Monodicalcium phosphate, Aliphos	--	72
Monodicalcium phosphate, others	73	65
Monocalcium phosphate, Aliphos	83	--
Monocalcium phosphate, others	76	64
Defluorinated phosphate (tricalcium phosphate + Ca Na P)	80	50
	Belgium	
Dicalcium phosphate	63	
Dicalcium phosphate dihydrate, Aliphos	73	
Dicalcium phosphate dihydrate, ex gelatine	--	
Monodicalcium phosphate, Aliphos	--	
Monodicalcium phosphate, others	--	
Monocalcium phosphate, Aliphos	92	
Monocalcium phosphate, others	--	
Defluorinated phosphate (tricalcium phosphate + Ca Na P)	--	
Holland: Grimbergen 1985, Mulder & Jongbloed 1985, Vandertol 1988, Vanderpeet		
Denmark: Foulum 1994		
Belgium: Eeckhout 1996		

#### Apparent digestibility with poultry; Holland and Belgium

Source	A	B	Average	Relative
Monocalcium phosphate, Aliphos	84	85	85	100
Monocalcium phosphate, others	76	-	76	90
Dicalcium phosphate dihydrate, Aliphos	77	83	80	94
Dicalcium phosphate dihydrate, bone grade	80	80	80	94
Dicalcium phosphate	53	70	62	73
Monodicalcium phosphate, others	79	-	79	94
Defluorinated phosphate	55	-	55	65

A: Simons and Van de Klis 1990-1994

B: Huyghebaert and DeGroote, 1996

Apparent digestibility of different types of  
feed phosphates, a summary

Source digestibility	Pigs		Poultry Phosphorus
	Phosphorus digestibility	Relative	
Dicalcium phosphate dihydrate, Aliphos	70	78	80
Dicalcium phosphate, Aliphos	65	72	70
Monocalcium phosphate, Aliphos	90	100	85
Monocalcium phosphate, others	80	89	76
Monodicalcium phosphate, Aliphos	80	89	83
Monodicalcium phosphate, others	75	83	75
Defluorinated phosphate	50-75	55-83	55
Source	Relative		
Dicalcium phosphate dihydrate, Aliphos	95		
Dicalcium phosphate, Aliphos	82		
Monocalcium phosphate, Aliphos	100		
Monocalcium phosphate, others	89		
Monodicalcium phosphate, Aliphos	98		
Monodicalcium phosphate, others	88		
Defluorinated phosphate	65		

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phytase? and lassen?	0

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**Search Results - Record(s) 1 through 10 of 10 returned.**☐ 1. Document ID: US 6291221 B1

L2: Entry 1 of 10

File: USPT

Sep 18, 2001

US-PAT-NO: 6291221

DOCUMENT-IDENTIFIER: US 6291221 B1

TITLE: Heat tolerant phytases

DATE-ISSUED: September 18, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
van Loon; Adolphus	Rheinfelden			CHX
Mitchell; David	Aesch			CHX

US-CL-CURRENT: 435/196; 435/252.3, 435/254.11, 435/320.1, 435/325, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWMC	Draw Desc	Image
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☐ 2. Document ID: US 6183740 B1

L2: Entry 2 of 10

File: USPT

Feb 6, 2001

US-PAT-NO: 6183740

DOCUMENT-IDENTIFIER: US 6183740 B1

TITLE: Recombinant bacterial phytases and uses thereof

DATE-ISSUED: February 6, 2001

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Short; Jay M.	Rancho Santa Fe	CA		
Kretz; Keith A.	San Marcos	CA		

US-CL-CURRENT: 424/94.6; 435/196, 536/23.2

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KWMC	Draw Desc	Image
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☐ 3. Document ID: US 6153418 A

L2: Entry 3 of 10

File: USPT

Nov 28, 2000

US-PAT-NO: 6153418  
DOCUMENT-IDENTIFIER: US 6153418 A

TITLE: Consensus phytases

DATE-ISSUED: November 28, 2000

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Lehmann; Martin	Inzlingen			DEX

US-CL-CURRENT: 435/195; 424/94.1, 424/94.6, 435/196

Full	Title	Citation	Front	Review	Classification	Date	Reference	Claims	KMIC	Draw Desc	Image
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☐ 4. Document ID: US 5985605 A

L2: Entry 4 of 10

File: USPT

Nov 16, 1999

US-PAT-NO: 5985605

DOCUMENT-IDENTIFIER: US 5985605 A

TITLE: DNA sequences encoding phytases of ruminal microorganisms

DATE-ISSUED: November 16, 1999

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cheng; Kuo Joan	Lethbridge			CAX
Selinger; Leonard Brent	Lethbridge			CAX
Yanke; Lindsey Jay	Lethbridge			CAX
Bae; Hee Dong	Seoul			KRX
Zhou; Luming	Salt Lake City	UT		
Forsberg; Cecil Wallace	Guelph			CAX

US-CL-CURRENT: 800/278; 435/196, 435/252.3, 435/252.31, 435/252.33, 435/254.11,  
435/254.23, 435/325, 435/419, 536/23.2, 536/23.7, 800/287, 800/288, 800/294,  
800/306

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KMIC	Draw Desc	Image
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☐ 5. Document ID: US 5939303 A

L2: Entry 5 of 10

File: USPT

Aug 17, 1999

US-PAT-NO: 5939303

DOCUMENT-IDENTIFIER: US 5939303 A

TITLE: Phytases of ruminal microorganisms

DATE-ISSUED: August 17, 1999

## INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Cheng; Kuo Joan	Lethbridge			CAX
Selinger; Leonard Brent	Lethbridge			CAX
Yanke; Lindsey Jay	Lethbridge			CAX
Bae; Hee Dong	Seoul			KRX
Zhou; Luming	Salt Lake City	UT		
Forsberg; Cecil Wallace	Guelph			CAX

US-CL-CURRENT: 435/196; 424/94.6, 426/61, 426/635, 435/183, 435/195

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 6. Document ID: EP 897985 A2

L2: Entry 6 of 10

File: EPAB

Feb 24, 1999

PUB-NO: EP000897985A2

DOCUMENT-IDENTIFIER: EP 897985 A2

TITLE: Consensus phytases

PUBN-DATE: February 24, 1999

## INVENTOR-INFORMATION:

NAME	COUNTRY
LEHMANN, MARTIN	DE

INT-CL (IPC): C12N 15/55; C12N 9/16; C12N 9/00; A23L 1/03; A23K 1/165; A61K 38/46  
EUR-CL (EPC): C12N009/16

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 7. Document ID: EP 897010 A2

L2: Entry 7 of 10

File: EPAB

Feb 17, 1999

PUB-NO: EP000897010A2  
DOCUMENT-IDENTIFIER: EP 897010 A2  
TITLE: Modified phytases

PUBN-DATE: February 17, 1999

## INVENTOR-INFORMATION:

NAME	COUNTRY
KOSTREWA, DIRK	DE
PASAMONTES, LUIS	CH
TOMSCHY, ANDREA	DE
VAN, LOON ADOLPHUS	CH
VOGEL, KURT	CH
WYSS, MARKUS	CH

INT-CL (IPC): C12N 15/55; C12N 9/16; C12N 1/15; C12N 1/19; A23K 1/165

Full	Title	Citation	Front	Review	Classification	Date	Reference
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KWIC	Draw Desc	Image
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☐ 8. Document ID: WO 9805785 A1

L2: Entry 8 of 10

File: EPAB

Feb 12, 1998

PUB-NO: WO009805785A1  
DOCUMENT-IDENTIFIER: WO 9805785 A1  
TITLE: PLANT PHYTASES AND BIOTECHNOLOGICAL APPLICATIONS

PUBN-DATE: February 12, 1998

## INVENTOR-INFORMATION:

NAME	COUNTRY
MAUGENEST, SEBASTIEN	FR
LESCURE, ANNE-MARIE	FR
PEREZ, PASCUAL	FR

INT-CL (IPC): C12N 15/55; C12N 15/82; A01H 5/00; A23K 1/165; C08B 30/00; C12P 19/04

EUR-CL (EPC): C12N009/16; C12N015/82, C12N015/82

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 9. Document ID: WO 9748812 A2

L2: Entry 9 of 10

File: EPAB

Dec 24, 1997



PUB-NO: WO009748812A2  
DOCUMENT-IDENTIFIER: WO 9748812 A2  
TITLE: DNA SEQUENCES ENCODING PHYTASES OF RUMINAL MICROORGANISMS

PUBN-DATE: December 24, 1997

## INVENTOR-INFORMATION:

NAME	COUNTRY
CHENG, KUO JOAN	CA
SELINGER, LEONARD BRENT	CA
YANKE, LINDSEY JAY	CA
BAE, HEE DONG	KR
ZHOU, LUMING	US
FORSBERG, CECIL WALLACE	CA

INT-CL (IPC): C12N 15/55; C12N 9/16; C12N 1/19; C12N 1/20; C12N 1/21; A01H 5/00;  
A23K 1/00; A23K 1/165; C12Q 1/44; C12Q 1/68; C12N 15/82  
EUR-CL (EPC): C12N009/16; A23K001/165, C12N015/82 , C12N015/82

Full	Title	Citation	Front	Review	Classification	Date	Reference
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☐ 10. Document ID: AU 200020928 A, WO 200043503 A1

L2: Entry 10 of 10

File: DWPI

Aug 7, 2000

DERWENT-ACC-NO: 2000-491161  
DERWENT-WEEK: 200055  
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TITLE: Novel phytases with improved properties such as temperature stability, pH stability and substrate specificity, for use in pharmaceuticals and compound foods and feeds

INVENTOR: LEHMANN, M

PRIORITY-DATA: 1999DK-0001340 (September 21, 1999), 1999DK-0000092 (January 22, 1999)

## PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 200020928 A	August 7, 2000		000	C12N009/16
WO 200043503 A1	July 27, 2000	E	238	C12N009/16

INT-CL (IPC): C12N 9/16

Full	Title	Citation	Front	Review	Classification	Date	Reference
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